

I Claim

1. A cell free extract from Ciliate phylum microorganism, wherein said cell free extract contains cholesterol desaturase activities selected from the group comprising Δ -7 and Δ -22 cholesterol desaturases activities that catalyze desaturation of cholesterol.

2. A cell free extract of Claim 1, wherein said cell free extract is selected from the group consisting of cell free homogenate, microsomal fraction and desaturase-enriched fraction, or a combination thereof, all from Ciliata phylum microorganism.

3. A cell free extract of Claim 1, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.

4. A process for manufacturing Δ 7 dehydrocholesterol (provitamin D3) and Δ 7,22 bis dehydrocholesterol comprising:

(a) mixing a cell free extract of claim 1 with a cholesterol substrate;

(b) incubating the mixture for a period of time enough to produce Δ 7 dehydrocholesterol and Δ 7,22 bis dehydrocholesterol;

(c) recovering said Δ 7 dehydrocholesterol and Δ 7,22 bis dehydrocholesterol by solvent extraction and chromatographic purification.

5. A substantial pure Δ^7 cholesterol desaturase enzyme from Ciliata phylum microorganism, wherein said enzyme is capable of catalyzing the conversion of a cholesterol substrate in Δ^7 dehydrocholesterol by introducing a double bound at the position seven in the cholesterol molecule.

6. A substantial pure Δ^7 cholesterol desaturase enzyme of Claim 5, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.

7. A substantial pure Δ^7 cholesterol desaturase enzyme according to claim 5, the enzyme

(a) having a molecular weight of approximately 60 kDa by gel chromatography;

(b) having an optimum pH range for enzymatic activity between 6.5-8.5;

(c) having an optimum temperature range for enzymatic activity of 28°C to 35°C;

(d) being unaffected by metal ions such as Ca^{+2} , Mn^{+2} and Mg^{+2} , EDTA concentrations and 2-mercaptoethanol;

(e) being inactivated after 1 minute at 100°C;

(f) being storage at -20°C by at least 6 months.

8. A substantial pure Δ^{22} cholesterol desaturase enzyme from Ciliata phylum microorganism, wherein said enzyme is capable of catalyzing the conversion of a cholesterol substrate in Δ^{22}

dehydrocholesterol by introducing a double bound at the position twenty-two in the cholesterol molecule.

9. A substantial pure $\Delta 22$ cholesterol desaturase enzyme of Claim 8, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.

10. A substantial pure $\Delta 22$ cholesterol desaturase enzyme according to claim 8, the enzyme

(a) having a molecular weight of approximately 60 kDa by gel chromatography;

(b) having an optimum pH range for enzymatic activity between 5.5-8.5;

(c) having an optimum temperature range for enzymatic activity of 28°C to 35°C;

(d) being unaffected by metal ions such as Ca^{+2} , Mn^{+2} and Mg^{+2} and EDTA concentrations;

(e) being inactivated after 1 minute at 100°C;

(f) being storage at -20°C by at least 6 months.

11. A process for preparing a substantial pure $\Delta 7$ cholesterol desaturase enzyme from Ciliata phylum microorganism according to claim 5, the process comprising the steps of:

(a) culturing a microorganism in a suitable medium, wherein said microorganism is capable of producing $\Delta 7$ cholesterol desaturases;

(b) disintegrating the culture and extracting the same with buffer solution containing, if necessary, non ionic surfactant or stabilizer as glycerol;

(c) subjecting the extract to a chromatography purification under suitable conditions; and

(d) eluting and recovering said $\Delta 7$ cholesterol desaturases.

12. The process according the claim 11, wherein the step of culturing is carried out in a medium containing 1% proteose peptone, 0.1% yeast extract, 0.5% glucose, 0.01% Sequestrene and 0,5mg% of 22 dehydrocholesterol.

13. The process according the claim 11, wherein the chromatography purification is selected from a group comprising size exclusion chromatography, anion exchange chromatography, cation exchange chromatography and combinations thereof.

14. A process for preparing a substantial pure $\Delta 22$ cholesterol desaturase enzyme from Ciliata phylum microorganism according to claim 8, the process comprising the steps of:

(a) culturing a microorganism in a suitable medium, wherein said microorganism is capable of producing $\Delta 22$ cholesterol desaturases;

(b) disintegrating the culture and extracting the same with buffer solution containing, if necessary, non ionic surfactant or stabilizer as glycerol;

(c) subjecting the extract to a chromatography purification on a suitable chromatography conditions; and

(d) eluting and recovering said $\Delta 22$ cholesterol desaturases.

15. The process according the claim 14, wherein the step of culturing is carried out in a medium containing 1% proteose peptone, 0.1% yeast extract, 0.5% glucose, 0.01% Sequestrene and 1.0 mg% of cholesterol.

16. The process according the claim 14, wherein the chromatography purification is selected from a group comprising size exclusion chromatography, anion exchange chromatography, cation exchange chromatography and combinations thereof.

17. The use of substantial pure $\Delta 7$ cholesterol desaturase enzyme from Ciliata phylum microorganism of claim 5 for producing $\Delta 7$ dehydrocholesterol (provitamin D3) employing cholesterol as substrate.

18. The use according the claim 17, wherein the cholesterol substrate es seleccionado del grupo comprendido por colesterol puro, cholesterol-containing products and cholesterol enriched fractions.

19. The use according the claim 17, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.

20. The use of pure Δ^7 cholesterol desaturase and substantial pure Δ^{22} cholesterol desaturase enzymes from Ciliata phylum microorganism of claims 5 and 8 for producing $\Delta^7,22$ bis dehydrocholesterol employing cholesterol as substrate.

21. The use according the claim 20, wherein the cholesterol substrate es seleccionado del grupo comprendido por colesterol puro, cholesterol-containing products and cholesterol enriched fractions.

22. The use according the claim 20, wherein the ciliate is selected from the group consisting of *Paremecium*, *Tetrahymena* and *Colpidium*.